Viroids and Obelisks

Those who benefit from sequence transparency should look in the mirror

ANANDAMIDE
2/3/2024

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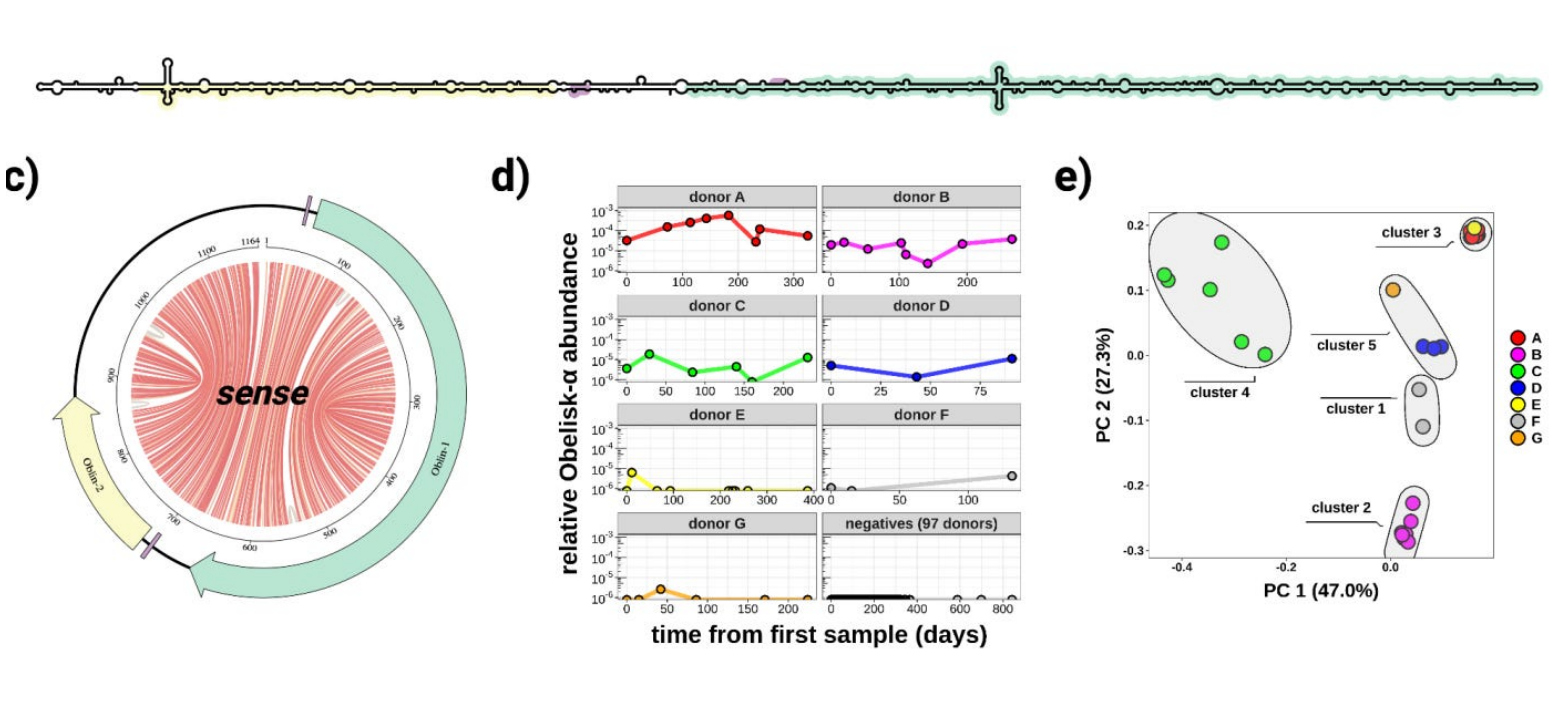


A new biological entity was discovered this week known as an Obelisk. This is a very important missing link between Viroids and Viruses.

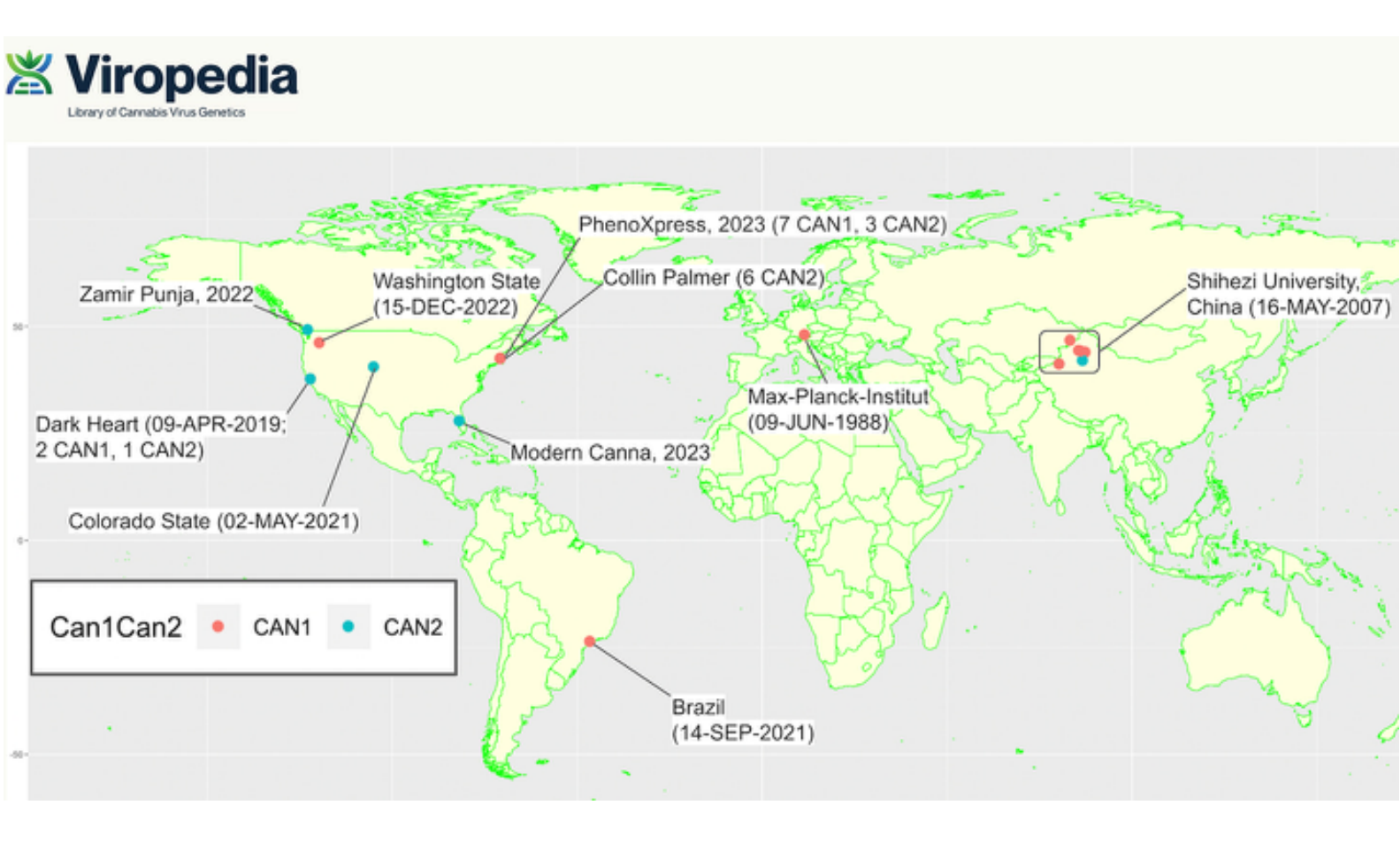
Followers of this stack know that we spend a lot of time studying Hop Latent Viroid that infects the cannabis plant. This is an excellent example that melts the minds of the 'no-virus' and 'PCR is useless' camp.

Why the melt down?

It is easy to chemically synthesize a pure circular RNA viroid of just 256 bases, infect a plant and watch your crop yield plummet 40%. With easy to clone plants, grows just PCR for the viroid, cull the positive plants and witness yield improvements that easily pay for the PCR costs.

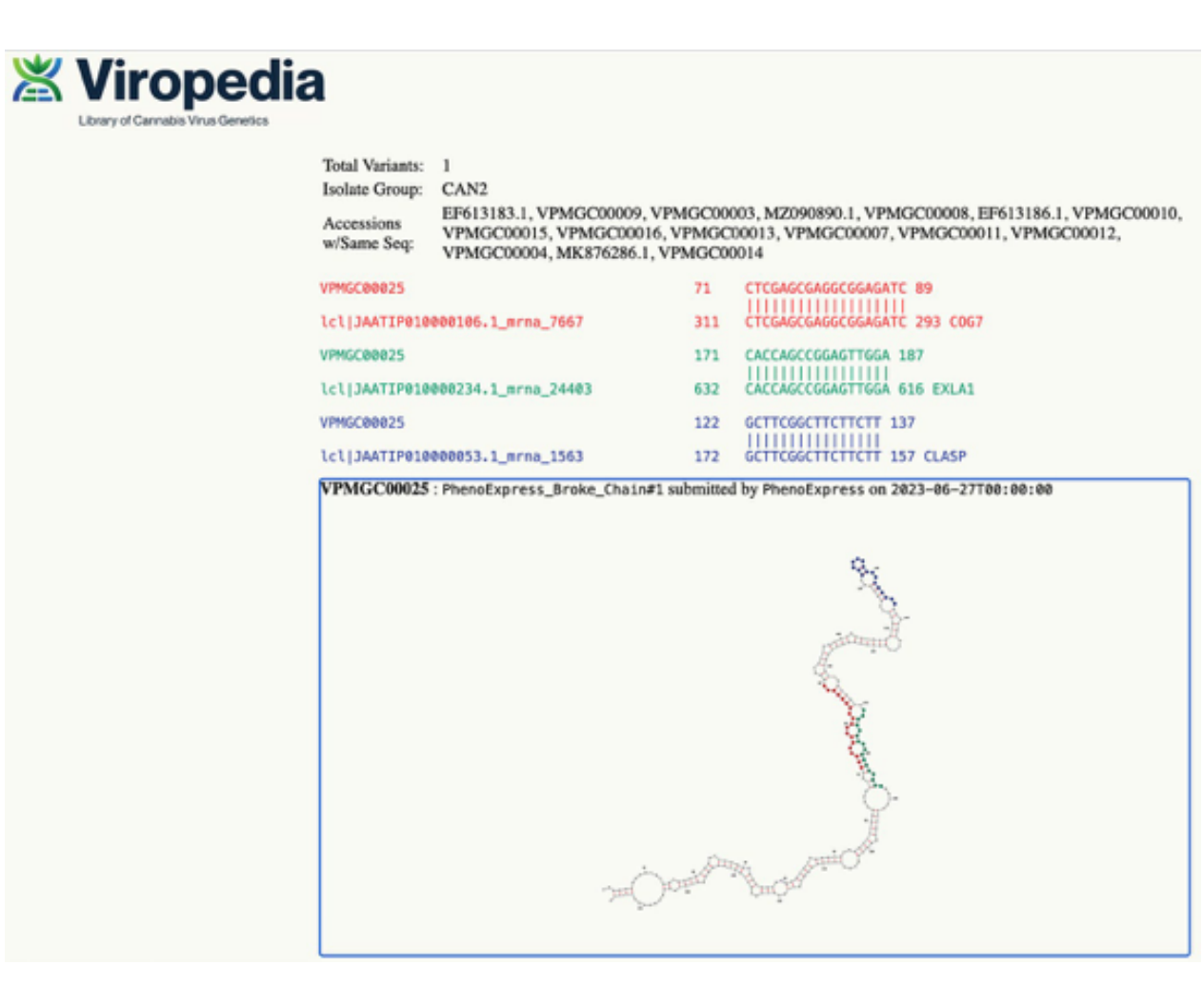
This viroid easily replicates in the host and is transmitted with mechanical (cloning scissors or insects) or pollen transmission. There is some speculation about fungal spore (Fusarium) and mycelium transmission in some viroids. This field is still nascent but either way, Koch's postulates is a slam dunk case and viroids are believed to evolutionary ancestors of viruses.

The 'no virus camp' is left with no where to go once you demonstrate you can isolate a pure viroid with an oligo synthesizer and it is infectious and causes transmissible disease. The 'No pandemic' folks are equally befuddled as Hop Latent Viroid has been documented in the US, Canada, China, Germany, Brazil and Israel despite no respiratory mode of transmission. It has a broader host range than initially named (Hop, Cannabis, Nettle, Tomato to name a few).


Viropedia
Library of Cannabis Virus Genetics
PhenoXpress, 2023 (7 CAN1, 3 CAN2)
Zamir Punja, 2022
Washington State (15-DEC-2022)
Collin Palmer (6 CAN2)
Shihezi University, China (16-MAY-2007)
Dark Heart (09-APR-2019; 2 CAN1, 1 CAN2)
Max-Planck-Institut (09-JUN-1988)
Modern Canna, 2023
Colorado State (02-MAY-2021)
Can1Can2
CAN1
CAN2
Brazil (14-SEP-2021)

The viroids have only been documented in plants. They are circular rod shaped RNAs (hairpins or complementary circles) that don't code for any proteins.

We track their sequence diversity and folding capacity at Viropedia.net.



Total Variants: 1
Isolate Group: CAN2
Accessions w/Same Seq: EF613183.1, VPMGC00009, VPMGC00003, MZ090890.1, VPMGC00008, EF613186.1, VPMGC00010, VPMGC00015, VPMGC00016, VPMGC00013, VPMGC00007, VPMGC00011, VPMGC00012, VPMGC00004, MK876286.1, VPMGC00014

```
VPMGC00025                          71   CTCGAGCGAGGCGGAGATC 89
                                         |||||||||||||||||||
lcl|JAATIP010000106.1_mrna_7667     311  CTCGAGCGAGGCGGAGATC 293 COG7
VPMGC00025                          171  CACCAGCCGGAGTTGGA 187
                                         |||||||||||||||||
lcl|JAATIP010000234.1_mrna_24403    632  CACCAGCCGGAGTTGGA 616 EXLA1
VPMGC00025                          122  GCTTCGGCTTCTTCTT 137
                                         ||||||||||||||||
lcl|JAATIP010000053.1_mrna_1563     172  GCTTCGGCTTCTTCTT 157 CLASP
```

VPMGC00025 : PhenoExpress_Broke_Chain#1 submitted by PhenoExpress on 2023-06-27T00:00:00

Alignments of Hop Latent Viroid genomes to cannabis genes. COG7, EXLA1 and CLASP are color coded on the viroid genome.

Their mechanism of action is believed to be through the down regulation of host mRNAs that share short (19-25bp) sequence homology of the viroid. This is process known as RNA interference (RNAi) which awarded Andrew Fire and others the Nobel Prize.

The reason to track the variants in the viroid genome is that occasionally a viroid SNP or a cannabis SNP induces a sequence homology between other host genes such as Callose Synthase . This gene codes for a protein that plugs the holes in the plasmodesmata of plant cell walls. By down regulating the mRNA for this gene, the cells become leaky and the viroid can spread cell to cell through the plasmodesmata pores.

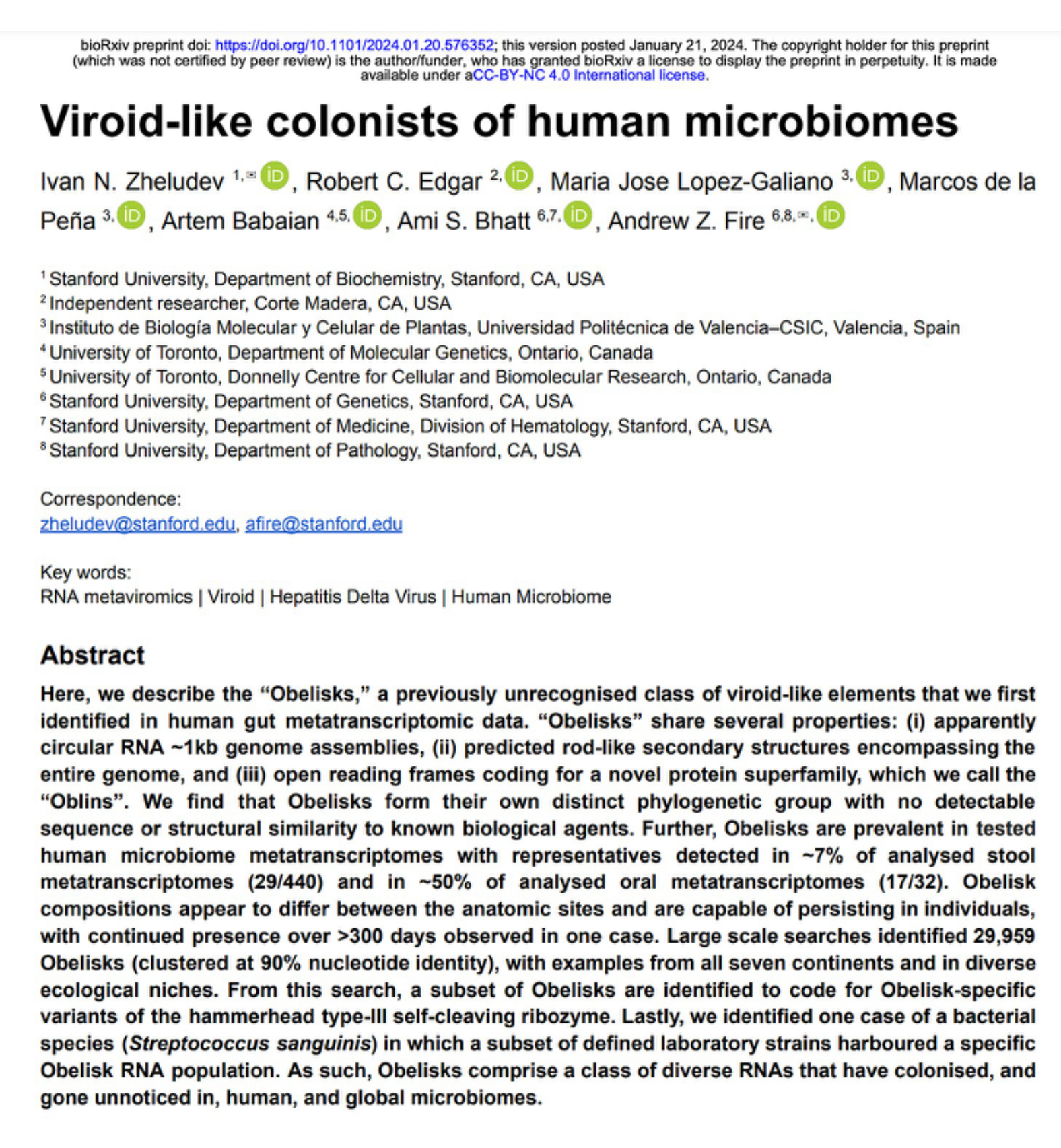
This same author who discovered RNAi has now discovered Obelisks and it may be as important of a discovery. Its important to realize they discovered this without lifting a single pipette. They simply had a hypothesis and went looking for it in the vast amount of public RNA-Seq data in the NCBI SRA. It shows you how important it is to bounce your hypothesis off of existing data in the public domain. Many people in the 'No-Pandemic' camp fail to do this on a regular basis and come up with all kinds of wacky hypothesis that are easily falsified by searching these data to see if your hypothesis still stands.

Here is an example of an excellent thread that disembowels many of the No-Pandemic Noise coming out of PANDA lately.

jrburns@umoja-research.com Subscribe

It's a long thread as there is infinite noise one can generate when you are too lazy to surf this data looking for a fit or nullification.

So what are Obelisks and how do they fit in next to Viroids and Viruses. Viroids don't code for any peptides. They just creates gobs of RNA once in the cell because circular RNAs amplify to extraordinary degrees due to a process known as Rolling circle amplification. Since the RNA is circular, the polymerases make long concatemers of single stranded RNA much like a fishing reel can churn out long lines of string.



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Viroid-like colonists of human microbiomes

Ivan N. Zheludev [1,✉], Robert C. Edgar [2], Maria Jose Lopez-Galiano [3], Marcos de la Peña [3], Artem Babaian [4,5], Ami S. Bhatt [6,7], Andrew Z. Fire [6,8,✉]

[1] Stanford University, Department of Biochemistry, Stanford, CA, USA
[2] Independent researcher, Corte Madera, CA, USA
[3] Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia–CSIC, Valencia, Spain
[4] University of Toronto, Department of Molecular Genetics, Ontario, Canada
[5] University of Toronto, Donnelly Centre for Cellular and Biomolecular Research, Ontario, Canada
[6] Stanford University, Department of Genetics, Stanford, CA, USA
[7] Stanford University, Department of Medicine, Division of Hematology, Stanford, CA, USA
[8] Stanford University, Department of Pathology, Stanford, CA, USA

Correspondence:
zheludev@stanford.edu, afire@stanford.edu

Key words:
RNA metaviromics | Viroid | Hepatitis Delta Virus | Human Microbiome

Abstract

Here, we describe the "Obelisks," a previously unrecognised class of viroid-like elements that we first identified in human gut metatranscriptomic data. "Obelisks" share several properties: (i) apparently circular RNA ~1kb genome assemblies, (ii) predicted rod-like secondary structures encompassing the entire genome, and (iii) open reading frames coding for a novel protein superfamily, which we call the "Oblins". We find that Obelisks form their own distinct phylogenetic group with no detectable sequence or structural similarity to known biological agents. Further, Obelisks are prevalent in tested human microbiome metatranscriptomes with representatives detected in ~7% of analysed stool metatranscriptomes (29/440) and in ~50% of analysed oral metatranscriptomes (17/32). Obelisk compositions appear to differ between the anatomic sites and are capable of persisting in individuals, with continued presence over >300 days observed in one case. Large scale searches identified 29,959 Obelisks (clustered at 90% nucleotide identity), with examples from all seven continents and in diverse ecological niches. From this search, a subset of Obelisks are identified to code for Obelisk-specific variants of the hammerhead type-III self-cleaving ribozyme. Lastly, we identified one case of a bacterial species (*Streptococcus sanguinis*) in which a subset of defined laboratory strains harboured a specific Obelisk RNA population. As such, Obelisks comprise a class of diverse RNAs that have colonised, and gone unnoticed in, human, and global microbiomes.

The cell then processes these concatemers but cutting them into pieces and re-circularizing them. With this much RNA in the cell, any other host mRNA that shares short sequences with the viroid RNA, gets down-regulated in a process known as RNA interference (also first described by Fire et al).

Some requirements for viroids

1)No ORF

2)Circular RNA

3)Rod-shaped or hairpins. Ie. self complementary

4)Usually 256-500 bases in length

Viruses, on the other hand, code for proteins. They are not necessarily circular, nor short or rod-shaped hairpins. The viruses usually have an encoded polymerase (RdRp) to help with the replication.

Viroids often have Ribozyme activity that helps to chop the concatemers into 256bp pieces for further circularization. Ribozymes are RNAs that fold into molecules that behave like catalytic enzymes.

Obelisks are in the middle. They are circular RNA, the are rod-shaped, usually under 1200bp BUT they contain short open reading frames and sometimes ribozymes. In this case they are called Oblin ORFs.

This is an important finding as it fills a hole in biology that was expected to exist but never found until aggressively looked for. They are easy to miss as short read assemblers (150bp reads) often get confused with tandem repeats that are longer than the read length of the sequencer.

To combat this the authors focused their search on RNA-Seq in the public domain that used stranded RNA-Seq. This retains more information on the origin of the strands of the RNA so that self complementary RNAs are more easy to detect and untangle. Circularity of the RNA also throws the assemblers for a loop as they are looking to make long linear chromosomes and are not trained to think about RNA that is circular.

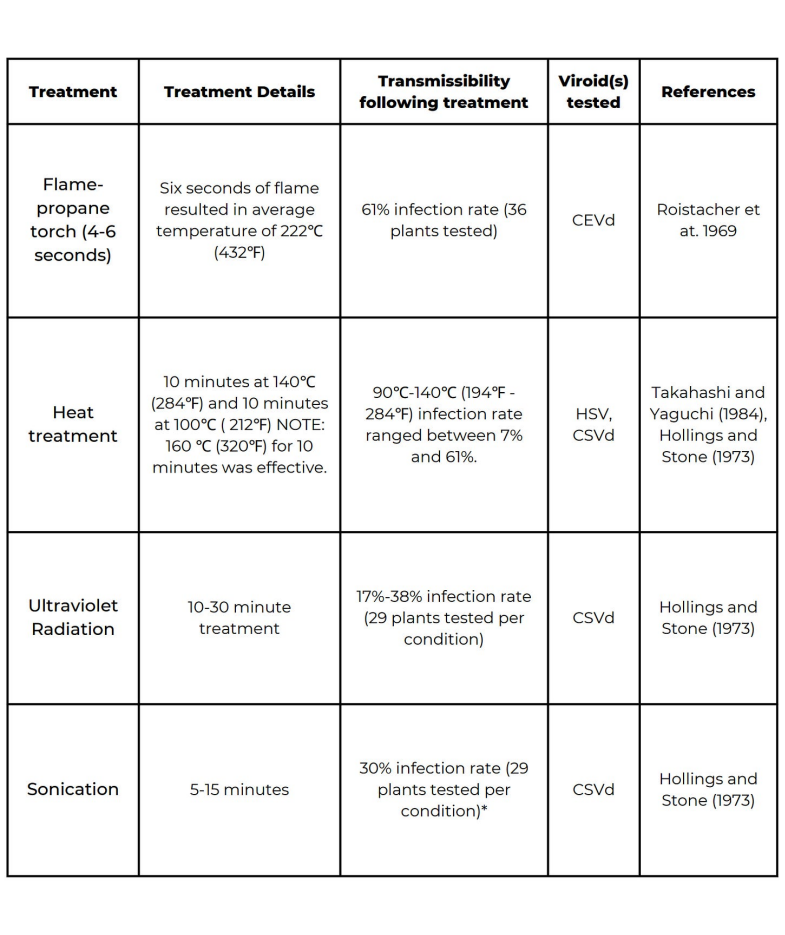
As a result, these structural RNAs have different selective pressures than just non-synonymous and synonymous mutations. They need to maintain Rod structure or some of the ribozyme activity of the RNA will be disturbed. Often they need to retain quadruplex Gs so typical codon analysis doesn't help to predict selective pressure.

These Obelisks are believe to replicate in bacteria in oral microbiomes of humans. Over 50% of healthy oral microbiomes had Obelisks.

The paper leaves many interesting questions in the discussion in terms of what these Obelisks do. Do they offer any clinical significance? How do they replicate if they can't be found in the DNA sequencing archives? How do they transmit? What is the function of the Oblin protein. Why the Hammerhead Ribozyme homology?

One thing is clear, the rod-shaped RNAs are incredibly sturdy. They found some patient with Obelisks present for over 300 days. This is expected as their evolutionary ancestors (viroids) are also Rod-shaped and are flame resistant.

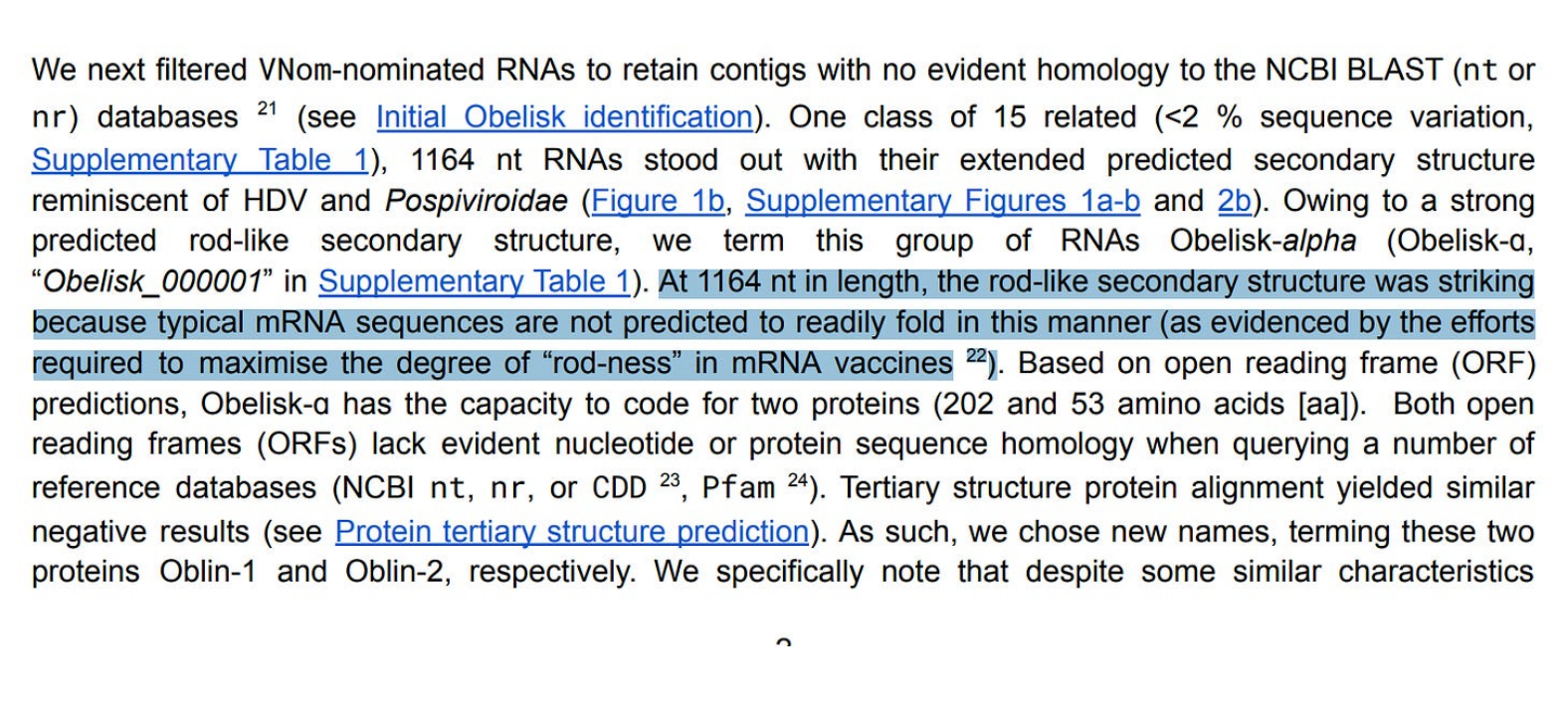
You don't often hear of flame resistance and RNA. Every lab tech is tortured over how delicate mRNA is but that delicacy is really just a reflection of the ubiquitous RNAses lab techs have on their hands.



Treatment	Treatment Details	Transmissibility following treatment	Viroid(s) tested	References
Flame-propane torch (4-6 seconds)	Six seconds of flame resulted in average temperature of 222℃ (432℉)	61% infection rate (36 plants tested)	CEVd	Roistacher et at. 1969
Heat treatment	10 minutes at 140℃ (284℉) and 10 minutes at 100℃ (212℉) NOTE: 160 ℃ (320℉) for 10 minutes was effective.	90℃-140℃ (194℉ - 284℉) infection rate ranged between 7% and 61%.	HSV, CSVd	Takahashi and Yaguchi (1984), Hollings and Stone (1973)
Ultraviolet Radiation	10-30 minute treatment	17%-38% infection rate (29 plants tested per condition)	CSVd	Hollings and Stone (1973)
Sonication	5-15 minutes	30% infection rate (29 plants tested per condition)*	CSVd	Hollings and Stone (1973)

Viroids can survive propane torches for 4-6 seconds and require bleach or RNaseIII to eliminate. The Obelisks likely share these properties.

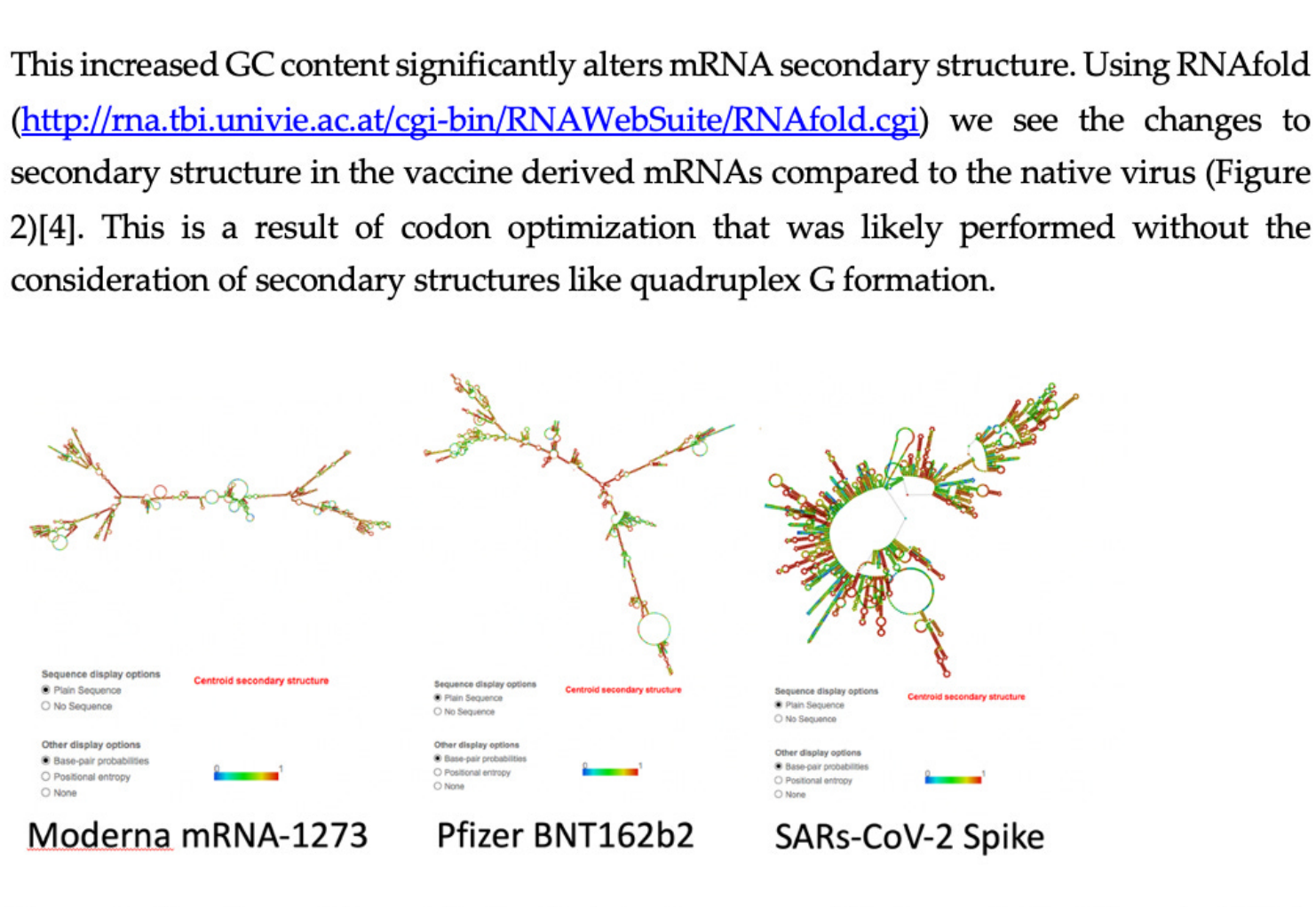
That led me to an interesting Easter Egg in the Obelisk paper.



We next filtered VNom-nominated RNAs to retain contigs with no evident homology to the NCBI BLAST (`nt` or `nr`) databases [21] (see Initial Obelisk identification). One class of 15 related (<2 % sequence variation, Supplementary Table 1), 1164 nt RNAs stood out with their extended predicted secondary structure reminiscent of HDV and *Pospiviroidae* (Figure 1b, Supplementary Figures 1a-b and 2b). Owing to a strong predicted rod-like secondary structure, we term this group of RNAs Obelisk-*alpha* (Obelisk-α, "*Obelisk_000001*" in Supplementary Table 1). At 1164 nt in length, the rod-like secondary structure was striking because typical mRNA sequences are not predicted to readily fold in this manner (as evidenced by the efforts required to maximise the degree of "rod-ness" in mRNA vaccines [22]). Based on open reading frame (ORF) predictions, Obelisk-α has the capacity to code for two proteins (202 and 53 amino acids [aa]). Both open reading frames (ORFs) lack evident nucleotide or protein sequence homology when querying a number of reference databases (NCBI `nt`, `nr`, or `CDD` [23], `Pfam` [24]). Tertiary structure protein alignment yielded similar negative results (see Protein tertiary structure prediction). As such, we chose new names, terming these two proteins Oblin-1 and Oblin-2, respectively. We specifically note that despite some similar characteristics

It turns out the mRNA vaccine experts are trying to leverage this rod-ness RNA structure in their codon optimizations to improve the longevity of the modRNA in humans.

We noted this back in 2021 when we put the vaccine mRNAs into RNAFold.

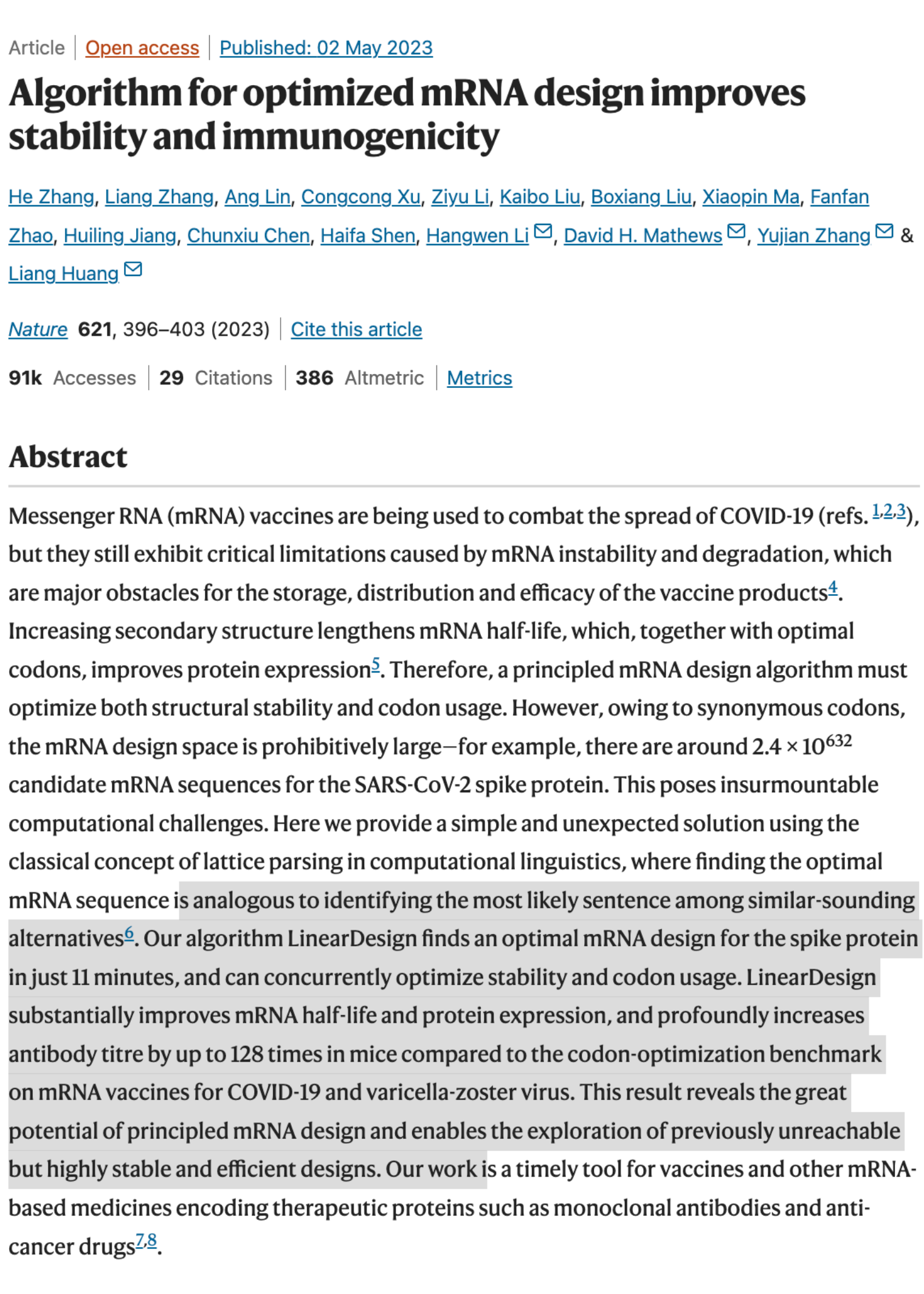


This increased GC content significantly alters mRNA secondary structure. Using RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) we see the changes to secondary structure in the vaccine derived mRNAs compared to the native virus (Figure 2)[4]. This is a result of codon optimization that was likely performed without the consideration of secondary structures like quadruplex G formation.

Figure 2. The three different Spike Protein Sequences (Moderna (left), Pfizer (middle), SARs-CoV-2(right)) analyzed with RNAfold.

While we noticed the GC enrichment and the Quadruplex G enrichment in the vaccines, I hadn't quite appreciated that the Rod-shapes they were delivering were deliberate.

Zhang et al explains how to do this.



Article | Open access | Published: 02 May 2023

Algorithm for optimized mRNA design improves stability and immunogenicity

He Zhang, Liang Zhang, Ang Lin, Congcong Xu, Ziyu Li, Kaibo Liu, Boxiang Liu, Xiaopin Ma, Fanfan Zhao, Huiling Jiang, Chunxiu Chen, Haifa Shen, Hangwen Li, David H. Mathews, Yujian Zhang & Liang Huang

Nature **621**, 396–403 (2023) | Cite this article

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Abstract

Messenger RNA (mRNA) vaccines are being used to combat the spread of COVID-19 (refs. [1,2,3]), but they still exhibit critical limitations caused by mRNA instability and degradation, which are major obstacles for the storage, distribution and efficacy of the vaccine products[4]. Increasing secondary structure lengthens mRNA half-life, which, together with optimal codons, improves protein expression[5]. Therefore, a principled mRNA design algorithm must optimize both structural stability and codon usage. However, owing to synonymous codons, the mRNA design space is prohibitively large—for example, there are around 2.4×10^{632} candidate mRNA sequences for the SARS-CoV-2 spike protein. This poses insurmountable computational challenges. Here we provide a simple and unexpected solution using the classical concept of lattice parsing in computational linguistics, where finding the optimal mRNA sequence is analogous to identifying the most likely sentence among similar-sounding alternatives[6]. Our algorithm LinearDesign finds an optimal mRNA design for the spike protein in just 11 minutes, and can concurrently optimize stability and codon usage. LinearDesign substantially improves mRNA half-life and protein expression, and profoundly increases antibody titre by up to 128 times in mice compared to the codon-optimization benchmark on mRNA vaccines for COVID-19 and varicella-zoster virus. This result reveals the great potential of principled mRNA design and enables the exploration of previously unreachable but highly stable and efficient designs. Our work is a timely tool for vaccines and other mRNA-based medicines encoding therapeutic proteins such as monoclonal antibodies and anti-cancer drugs[7,8].

Once again we have molecular biologists obsessed with this thinking that longer duration must be better.

Ooops.

This is yet another reason why the modRNAs are being detected for months after vaccination despite being told they should clear in 48 hours.

This is an amazing discovery that is as significant as the discovery of LincRNAs. I have had the pleasure of publishing with Andrew Fire with the SOLiD sequencer. This was one of the first publications using the SOLiD sequencer and it took the cover of Genome Research in 2008.

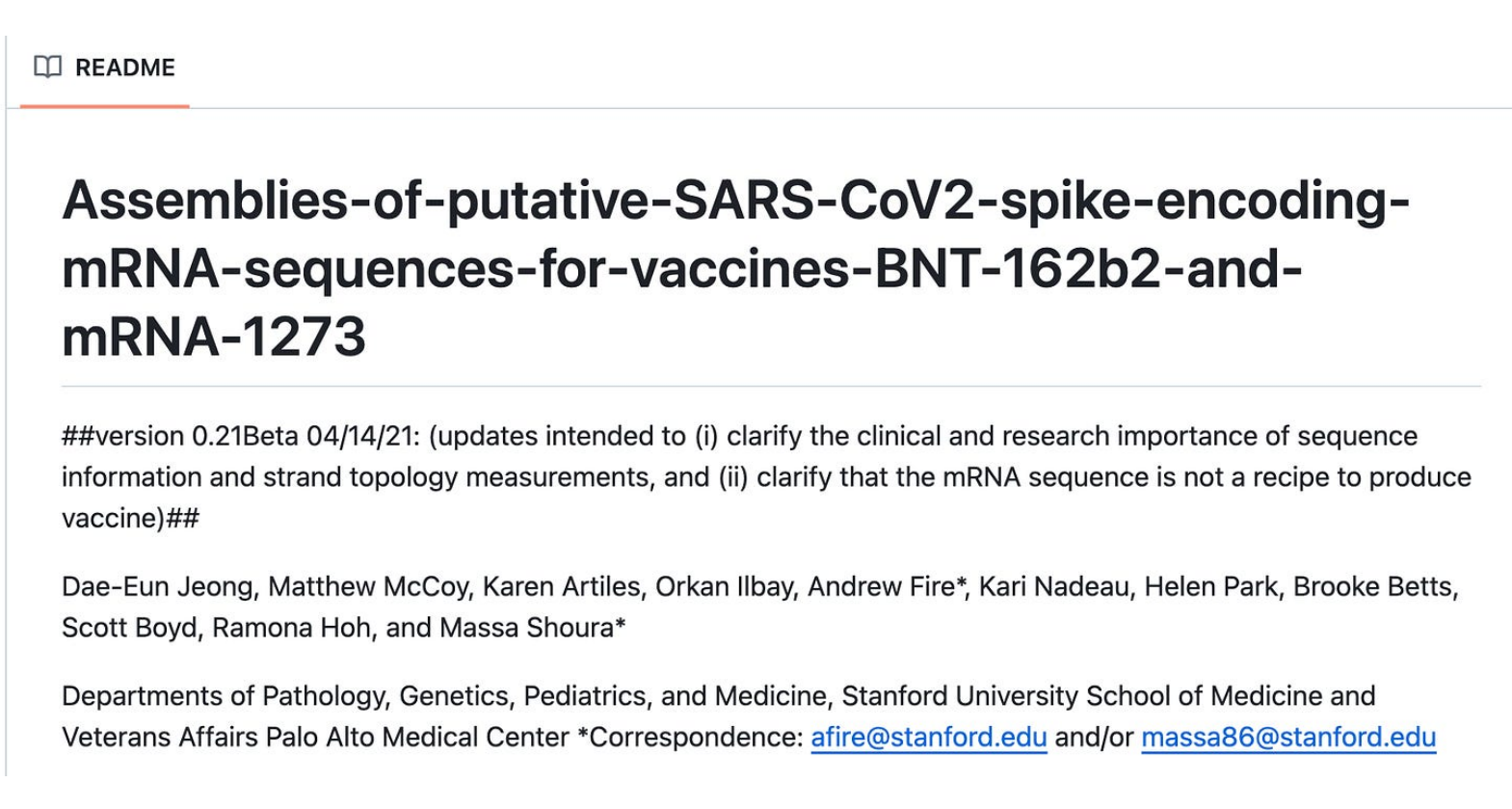
ISSN 1088-9051
July 2008
GENOME RESEARCH
Volume 18 Number 7
Nucleosome Positioning • Human Skin Microbiota • Translocation Breakpoint Mapping by Next-Generation Sequencing • Repeat Expansion Diseases • Amphioxus Genome Insights into Vertebrate Origins
Cold Spring Harbor Laboratory Press

Despite all of the Fire's labs accomplishments, I have one critique. This discovery is a prime example of the importance of submitting raw reads from your sequencing projects to the SRA. This affords discoveries likes this that are not apparent until data can be mined from diverse projects not focused on these interesting post hoc questions.

You may recognize the Fire labs name from when they sequenced the vaccines and **DIDNT** put such raw read sequence public despite frequent requests to do so.

Had they put them public the vaccine plasmid DNA contamination would have been known 2-3 years earlier!

This is not normal. Most genome projects and journals require the raw reads to be put public for exactly this reason. Other researches mine the data and find many diamonds in the rough that may alter the conclusions. Steve Massey and Steve Quay have found all kinds of interesting details inside these SRA archives that have informed on the origins of the C19 virus.



README

Assemblies-of-putative-SARS-CoV2-spike-encoding-mRNA-sequences-for-vaccines-BNT-162b2-and-mRNA-1273

##version 0.21Beta 04/14/21: (updates intended to (i) clarify the clinical and research importance of sequence information and strand topology measurements, and (ii) clarify that the mRNA sequence is not a recipe to produce vaccine)##

Dae-Eun Jeong, Matthew McCoy, Karen Artiles, Orkan Ilbay, Andrew Fire*, Kari Nadeau, Helen Park, Brooke Betts, Scott Boyd, Ramona Hoh, and Massa Shoura*

Departments of Pathology, Genetics, Pediatrics, and Medicine, Stanford University School of Medicine and Veterans Affairs Palo Alto Medical Center *Correspondence: afire@stanford.edu and/or massa86@stanford.edu

I repeated these requests for the raw reads even after the plasmid contamination was public. Still no response. This is very suspect. It implies their reads have the evidence of the plasmids and they don't want the world to know they buried that data in the name of preventing 'vaccine hesitancy'.

It is this White Lab Coat paternalism that infects the minds that justify a 'Nobel Lie'. We must save the Plebs from themselves and hide data. They can't handle the truth or they will brew misinformation while they shove rocks up their nose.

YOU CAN'T HANDLE THE TRUTH!

Its this ivory tower disdain for public intellect that always leads to more authoritarianism and political centralization of power. Since the public cant be trusted with the truth, we need government to mandate what we know to be best for them.

I suspect their lab will downplay the significance of the plasmid DNA as being too short to matter. This will be interesting given their paper currently focuses on the functionality of short sequences like viroids and Obelisks.

This should be a reminder that Codon Optimization is a misnomer. Our current understanding of the complexity of biology leaves only those lacking humility to name it as such. Codon Hopetimization is far more appropriate given changes in RNA secondary structure can have such a large impact on RNA processing and RNA interference. The desire to usher in a new platform for a liability free money machine has led Icarus to the sun like a moth to a flame.

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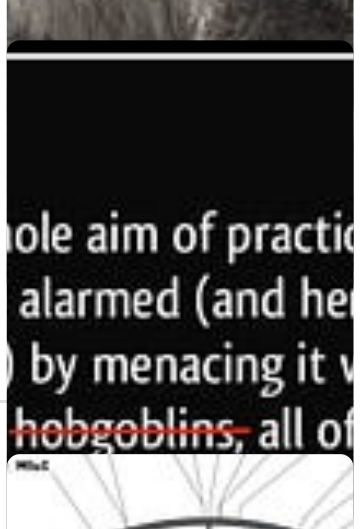
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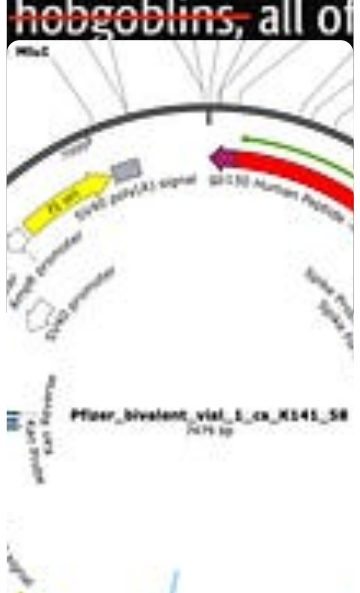

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